

In re Patent Application of:

**Daniell**

Serial No.: **10/520,204**

Filed: **08/26/2005**

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### **In the Claims**

Please cancel the indicated claims without prejudice and substitute the pending claims as set forth below in a complete listing. Language added is shown underlined and language deleted is shown in strike through or enclosed in brackets. The amendments include no new matter and are fully supported in the application as filed.

1.(previously presented) A transformation vector effective for stably transforming a plastid genome, said vector comprising, as operably-linked components, a first flanking sequence, at least one DNA sequence coding for a mercuric ion reductase (merA) and an organomercurial lyase (merB), a sequence encoding an antibiotic-free selectable marker, and a second flanking sequence.

2.(previously presented) The vector of claim 1, wherein said at least one DNA sequence is a phyto remediation operon.

3.(original) The vector of claim 1 or 2 further comprising a regulatory sequence.

4.(original) The vector of claim 3, wherein said regulatory sequence comprises a promoter operative in said plastid genome.

5.(original) The vector of claim 4, wherein said promoter is 16srRNA.

6.(original) The vector of claim 3, wherein said regulatory sequence comprises a 3' untranslated region (UTR).

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7.(previously presented) The vector of claim 1, wherein the vector is competent for stably integrating in the plastid genome of a plant species and wherein the flanking DNA sequences are substantially homologous to sequences in a spacer region of said plastid genome.

8.(original) The vector of claim 7, wherein said spacer region is a transcriptionally active spacer region.

9.(currently amended) The vector of claim 1, wherein the plastid is selected from a chloroplast, a chromoplast, an amyloplast, a ~~proplastide~~ proplastid, a leucoplast and an etioplast.

10.(cancelled)

11.(original) The vector of claim 1, wherein said first flanking sequence is trnI, and wherein said second flanking sequence is trnA.

12.(original) The vector of claim 11, wherein trnI and trnA provide for homologous recombination to insert an operon coding for a protein suitable for inactivating a contaminant compound into the spacer region in an inverted repeat region of a chloroplast genome.

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13.(currently amended) The vector of claim 1, wherein said vector is stably integrated into a plastid genome and said vector is located in a single copy region of said plastid genome.

14.(previously presented) The vector of claim 6, wherein said 3' UTR is a 3' UTR of psbA.

15-16.(cancelled)

17.(previously presented) The vector of claim 1, wherein said antibiotic-free selectable marker is betaine aldehyde dehydrogenase (BADH).

18-19.(cancelled)

20.(currently amended) A method for producing at least one ~~DNA sequence coding for a~~ protein effective for inactivating a contaminant compound, the method comprising:  
integrating the ~~plastid~~ transformation vector of claim 1 into the plastid genome of a plant cell; and  
growing said plant cell to thereby express said at least one DNA sequence.

21.(currently amended) The method of claim 20, wherein said at least one DNA sequence ~~coding for a protein suitable for inactivating a contaminant compound~~ is competent for the plant cell to phyto remediate a contaminant compound.

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22.(original) A plant stably transformed with the transformation vector of claim 1.

23.(previously presented) A progeny of the plant of claim 22, said progeny being stably transformed with said vector.

24.(previously presented) A seed of the plant of claim 22, the seed containing said vector.

25 (previously presented) A plant part of the plant of claim 22, the plant part containing said vector.

26.(previously presented) A plant that comprises at least one chloroplast transformed with the vector of claim 1.

27.(previously presented) The plant of claim 26, wherein said plant further comprises a plurality of said chloroplasts in mature leaves.

28.(previously presented) The plant of claim 26, wherein said plant further comprises a plurality of said chloroplasts in young leaves.

29.(previously presented) A plastid transformation vector effective for stably transforming a plastid genome, comprising, as operably-linked components, a first flanking sequence capable of integrating said plastid transformation vector into the plastid genome, an operon comprising *merA* and *merB* genes, a sequence encoding a

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marker selected from an antibiotic-free marker and an antibiotic resistance marker, and a second flanking sequence capable of integrating said plastid transformation vector into the plastid genome.

30.(original) The plastid transformation vector of claim 29, wherein said first and second flanking sequences allow site-specific integration of the operon containing the *merA* and *merB* genes into an inverted repeat region of the plastid genome between *tmI* (tRNA Ile) and *trnA* (tRNA Ala) genes.

31.(currently amended) The plastid transformation vector of any one of claims 29 or 30, wherein said sequence encoding a marker is selected to be an antibiotic-resistance marker and wherein said operon further comprises an *aadA* gene as the selectable marker.

32.(previously presented) The plastid transformation vector of claim 29, further comprising a 3' untranslated region(3' UTR) positioned downstream of the operon, and upstream of said second flanking sequence.

33.(previously presented) The plastid transformation vector of claim 32, wherein said 3' UTR is from a *psbA* chloroplast gene.

34.(previously presented) A method of detoxifying mercury, said method comprising integrating the vector of claim 1 into a plastid genome of a plant cell, culturing said plant cell to express *merA* and *merB*, and exposing said plant cell to mercury.

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35.(previously presented) The vector of claim 2, wherein the operon is the *merAB* operon.

36.(currently amended) A plant cell containing a plastid ~~including~~ which includes an expression cassette having as operably linked components[,] a promoter functional in said plastid, a *merAB* operon, a transcription termination region, a sequence encoding an antibiotic-free selectable marker, and DNA sequences flanking the expression cassette and effective for stably integrating said expression cassette into a genome of said plastid.

37-40.(cancelled)